

Claims :

We claim

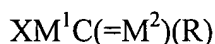
1. An automated method of determination of metabolic stability of library of compounds from cytochrome P450 enzyme reactions by isotope dilution mass spectrometric analysis comprising the steps of :
 - a) converting a portion of each said library compound into a stable isotope labeled internal standard by addition of a stable isotope labeled chemical reagent; and
 - b) converting another portion of each said library compound into a compound of identical structure, with the exception of the stable isotope atoms, as that of said stable isotope labeled internal standard comprising addition of a non-isotope version of said stable isotope reagent; and
 - c) separating said stable isotope labeled internal standards and said non-isotope labeled converted compounds by an extraction method; and
 - d) incubating other portions of said library of compounds in the presence of cytochrome P450 enzyme systems at body temperature for periods of time; and
 - e) adding quenching chemical reagents to terminate said enzyme reactions; and
 - f) adding said stable isotope labeled internal standards to said terminated reactions; and
 - g) adding said non-isotope version of said stable isotope labeled chemical reagent to said terminated reactions to convert the remaining of each said library compound into said non-isotope labeled converted compounds; and
 - h) separating said converted compound and its internal standard from said reactions by an extraction method; and
 - i) determining the molecular ions of said stable isotope labeled internal standards and said non-isotope labeled converted compounds; and
 - j) determining the most abundant daughter ions of said stable isotope labeled internal standards and said non-isotope labeled converted compounds; and
 - k) determining the ion ratio of said converted compound to said corresponding internal standard for each of said enzyme reactions using tandem mode of mass spectrometric analysis; and
 - l) determining the percent remaining of each said library compound from said enzyme reactions from said ion ratios.
2. The method of claim 1 wherein said steps a), b), and d) are performed concurrently.
3. The method of claim 1 wherein said extraction in steps c) and h) can be any appropriate separating methods such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction.

4. The method of claim 1 wherein said sample contains either a singularity or a plurality of each class of said library compounds.
5. The method of claim 1 wherein said library compounds in step a) are converted to said internal standards using a single isotope labeled chemical reagent.
6. The method of claim 1 wherein said library compounds in steps b) and g) are converted to compounds of identical structure as that of said internal standards, except the labeled atoms, using a single non-labeled version of said chemical reagent.
7. The method of claim 1 wherein said conversion in steps a), b) and g) are 100% quantitative.
8. The method of claim 1 wherein the converting step g) is performed before the extraction step h).
9. The method of claim 1 wherein said quenching chemical reagent is an organic solvent.
10. The method of claim 1 wherein said library compound has the formula



wherein X is H, F, Cl, Br, I, N₃, OR¹, SR¹, CN, NR¹ R², a cyclic group, a substituted cyclic group, a heterocyclic group, a substituted heterocyclic group, a linear branched alkyl group of 1 to 20 carbons, a substituted linear or branched alkyl group, an alkenyl group, a substituted alkenyl group, an alkynyl group, a substituted alkynyl group, an acyl group, a substituted acyl group, wherein R¹ and R² are each independently the same or different linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M is NH, O, CO, and wherein R is H, OH, a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group.

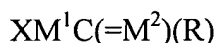
11. The method of claim 1 wherein said converted compound for mass spectrometric analysis has the formula



wherein X is H, F, Cl, Br, I, N₃, OR¹, SR¹, CN, NR¹ R², a cyclic group, a substituted cyclic group, a heterocyclic group, a substituted heterocyclic group, a linear branched alkyl group of 1 to 20 carbons, a substituted linear or branched alkyl group, an alkenyl group, a substituted alkenyl group, an alkynyl group, a substituted alkynyl group, an acyl group, a substituted acyl group, wherein R¹ and R² are each independently the same or different linear or branched alkyl group, a

substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M^1 is N, O, CH, CR^3 , wherein R^3 is a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M^2 is O or NOR^4 , and wherein R^4 is an alkyl group, and wherein R is H, NR^5 , SR^5 , R^5 , OR^5 , NHR^5 a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein R^5 is an alkyl group.

12. The method of claim 1 wherein said stable isotope labeled internal standard for mass spectrometric analysis has the formula



wherein X is H, F, Cl, Br, I, N_3 , OR^1 , SR^1 , CN, NR^1 , R^2 , a cyclic group, a substituted cyclic group, a heterocyclic group, a substituted heterocyclic group, a linear branched alkyl group of 1 to 20 carbons, a substituted linear or branched alkyl group, an alkenyl group, a substituted alkenyl group, an alkynyl group, a substituted alkynyl group, an acyl group, a substituted acyl group, wherein R^1 and R^2 are each independently the same or different linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M^1 is N, O, CH, CR^3 , wherein R^3 is a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein M^2 is O or NOR^4 , and wherein R^4 is a stable isotope labeled alkyl group, and wherein R is H, NR^5 , SR^5 , R^5 , OR^5 , NHR^5 a linear or branched alkyl group, a substituted linear or branched alkyl group, a substituted cyclic alkyl group, an acyl group, a substituted acyl group, and wherein R^5 is a stable isotope labeled alkyl group.

13. The method of claim 1 wherein the stable isotope labeled chemical reagent is selected from a group consisting of a stable isotope labeled acid anhydride, a stable isotope labeled acid chloride, a stable isotope labeled chloroformate, a stable isotope labeled isocyanate, a stable isotope labeled thioisocyanate, a stable isotope labeled alkoxyamine, a stable isotope labeled alkylhydrazine.
14. The method of claim 1 wherein the non-isotope version of said stable isotope labeled chemical reagent is selected from a group consisting of an acid anhydride, an acid chloride, a chloroformate, an isocyanate, a thioisocyanate, an alkoxyamine, and an alkylhydrazine
15. The method of claim 1 wherein said cytochrome P450 enzyme systems are cryopreserved or fresh human and animal hepatocytes, microsomes, S9 fractions or solutions containing individual cytochrome P450 isoenzymes.